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Effect of Oral Treatment with Hexadecyloxypropyl-[(*S*)-9-(3-Hydroxy-2-Phosphonylmethoxypropyl)Adenine] [(*S*)-HPMPA] or Octadecyloxyethyl-(*S*)-HPMPA on Cowpox or Vaccinia Virus Infections in Mice[▽]

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We have previously reported that (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine, or (*S*)-HPMPA, is active in vitro against cowpox virus (CV) and vaccinia virus (VV) but is not active orally in animals. However, the ether lipid esters of (*S*)-HPMPA, hexadecyloxypropyl-[(*S*)-HPMPA] [HDP-(*S*)-HPMPA] and octadecyloxyethyl-[(*S*)-HPMPA] [ODE-(*S*)-HPMPA], had significantly enhanced activity in vitro and are orally bioavailable in mice. In the current study, HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA were prepared in water and administered once daily by oral gavage to mice at doses of 30, 10, and 3 mg/kg of body weight for 5 days beginning 24, 48, or 72 h after inoculation with CV or VV. Oral HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA were both highly effective ($P < 0.001$) at preventing mortality due to CV at 30 mg/kg, even when treatments were delayed until up to 72 h postinfection. ODE-(*S*)-HPMPA or HDP-(*S*)-HPMPA were also highly effective ($P < 0.001$) at preventing mortality in mice infected with VV at 30 mg/kg when treatments were delayed until to 48 or 72 h postinfection, respectively. Protection against both viruses was associated with a significant reduction of virus replication in the liver, spleen, and kidney but not in the lung. These data indicate that HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA are active when given orally against lethal CV and VV infections in mice, and further evaluation is warranted to provide additional information on the potential of these orally active compounds for treatment of human orthopoxvirus infection.

Although cidofovir (CDV) has been approved as an investigational new drug for emergency use in orthopoxvirus infections in humans, its lack of activity when given orally and its potential for causing nephrotoxicity limits its usefulness (15). A modification to CDV has resulted in a compound (hexadecyloxypropyl [HDP]-CDV) that is very active when given orally and provides excellent protection against orthopoxvirus infections in mice (5, 22). HDP-CDV is currently in phase 1 clinical studies, and its potential for use in humans is not yet established.

(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [(*S*)-HPMPA] was synthesized and described as an antiviral as early as 1986, and its antiviral properties (10, 11, 12, 25) and pharmacokinetic profile (4, 7) were determined. It has broad-spectrum activity against double-stranded DNA viruses, such as herpesviruses and orthopoxviruses, and was effective both in vitro and in vivo (1, 2, 9, 10, 12, 13, 14, 15). Studies attempting to show in vivo activity of (*S*)-HPMPA have utilized parenteral dosing, suggesting that the compound, like CDV (HPMPC), is not orally active (4, 23). The synthesis and evaluation of alkoxyalkylesters of CDV and (*S*)-HPMPA have shown these compounds to be highly active when given orally and effective in vitro against both orthopoxviruses and cytomegaloviruses (CMV) (3, 5, 6, 14, 18, 22). Experiments conducted with animal models confirmed the activity for HDP-CDV against human and murine CMV (16), cowpox virus (CV), and vaccinia virus (VV) (5, 17, 22), and these results have been useful for helping to predict potential therapies for human use in the event of a bioterrorist release of smallpox virus (20).

As with most infectious agents, the natural emergence of drug resistance or the intentional genetic manipulation to create drug-resistant variants by bioterrorists is possible. An orally available drug combination for treatment of orthopoxvirus infections could alleviate some of these concerns, particularly if delayed treatments are effective. The two potential therapies to date in development for treatment of smallpox in humans are HDP-CDV (CMX001) (5, 20, 22) and ST-246 (27), but additional therapies are needed. The purpose of the present experiments was to determine the oral bioavailability and pharmacokinetics of HDP-(*S*)-HPMPA and octadecyloxyethyl-[(*S*)-HPMPA] [ODE-(*S*)-HPMPA] in mice and evaluate the ability of these new molecules to protect mice inoculated with VV or CV.

MATERIALS AND METHODS

Chemistry. HDP-(*S*)-HPMPA and ODE-(S)-HPMPA were synthesized as reported previously (3) by using the method outlined in Fig. 1.

Briefly, the compounds were prepared by alkylation of N^6,*O*-bistrityl (*S*)-2,3-dihydroxypropyladenine (NaH, triethylamine) with HDP and ODE monoesters of toluenesulfonyloxymethyl phosphonate, which, in turn, were obtained from diethyl toluenesulfonate and the appropriate lipid alcohol. Deprotection with

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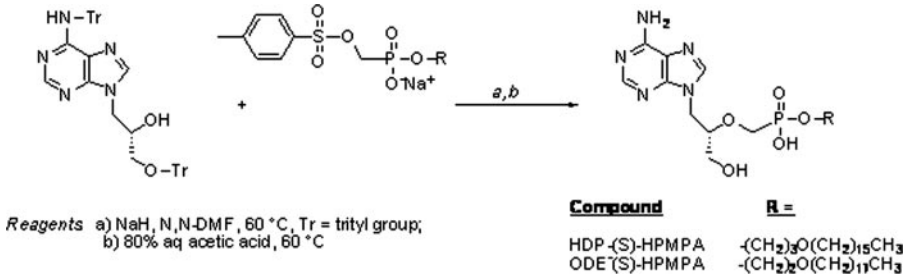

FIG. 1. Synthesis of HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA. aq, aqueous.

80% aqueous acetic acid gave the (*S*)-HPMPA analogs. The final compounds were fully characterized by ^{1}H nuclear magnetic resonance, ^{31}P nuclear magnetic resonance, mass spectroscopy, and elemental analysis. Full details of the synthesis have been reported previously (3). HDP-[8-^{14}C](*S*)-HPMPA was synthesized by Moravek Biochemicals, Inc. (Brea, CA) by using the method shown in Fig. 1.

Pharmacokinetics. Nonfasted female Swiss Webster mice weighing approximately 25 g each received a single dose of HDP-(*S*)-[8-^{14}C]-HPMPA in 0.7% saline, at 10 mg/kg of body weight, by oral gavage or by intraperitoneal (i.p.) injection. The mice were euthanized at 1, 3, 6, 12, and 24 h for blood collection into heparinized tubes. The plasma was centrifuged, 50 μl of plasma was added to 10 ml of Ecolite (MP Biomedical, Irvine, CA), and the plasma contents of the drug and metabolites were determined by liquid scintillation counting. The data are expressed as total micromoles of HDP-[8-^{14}C]-(*S*)-HPMPA and metabolites per liter of plasma (μM). Data for each time point are the averages from three replicates.

In vitro efficacy and toxicity. The activity and toxicity of CDV, (*S*)-HPMPA, HDP-(*S*)-HPMPA, and ODE-(*S*)-HPMPA in vitro were determined with human foreskin fibroblast (HFF) cells using plaque reduction methods as described previously (3, 18). Briefly, to determine efficacy, confluent monolayers of HFF cells in six-well plates were infected with VV or CV and then treated with various concentrations of drug for 3 days at 37°C. Toxicity was evaluated using uninfected confluent monolayers of HFF cells seeded in 96-well plates incubated with various concentrations of drug for 7 days at 37°C. The values are the concentrations which cause cytotoxic effects on 50% of cells (CC_{50}) or the concentrations which reduce viral replication by 50% (EC_{50}s) and are the means from two or more assays ± standard deviations.

Viruses. The VV strain Copenhagen and the CV strain Brighton were kindly provided by John W. Huggins (U.S. Army Medical Research Institute of Infectious Disease, Frederick, MD). The VV strain WR was obtained from the American Type Tissue Collection (ATCC), Manassas, VA. Stock virus pools were propagated in Vero cells, also obtained from ATCC.

Mice. Female BALB/c mice, 3 weeks of age, were obtained from Charles River Laboratories, Raleigh, NC. Mice were group housed in microisolator cages and utilized at a quantity of 15 mice per treatment group for efficacy and pathogenesis studies. Mice were obtained, housed, utilized, and euthanized according to USDA and AAALAC regulatory policies. All animal procedures were approved by the Institutional Animal Care and Use Committee, University of Alabama at Birmingham, prior to the initiation of studies.

Female Swiss Webster mice were obtained from Charles River Laboratories, Raleigh, NC. Mice were group housed in microisolator cages. Mice were obtained, housed, utilized, and euthanized according to USDA and AAALAC regulatory policies for pharmacokinetic studies. All animal procedures were approved by the Institutional Animal Care and Use Committee, University of California at San Diego, prior to the initiation of studies.

Antiviral compounds. CDV (Vistide; Gilead Sciences, Foster City, CA) was diluted in sterile saline to yield the desired doses within a 0.1-ml volume. It was administered i.p. once daily for 5 days. HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA were provided as dry powders and were weighed and dissolved in deionized water to yield the desired doses within a 0.2-ml volume for oral gavage. Each was administered orally once daily for 5 days. Uninfected mice served as toxicity controls for each compound and were treated similarly. Mortality resulting from compound administration in uninfected mice is considered toxicity.

Experimental infections and viral pathogenesis. VV WR and CV infections were initiated by intranasal inoculation of anesthetized (ketamine-xylazine) BALB/c mice (21, 22) with an approximate 90% lethal dose. CV (3.3×10^4 to 9×10^5 PFU/animal) or VV WR (1×10^4 PFU/animal) was instilled into both nostrils by using a micropipettor, with a total volume of 40 μl per animal. For the efficacy experiments with animal death as an endpoint, mice were checked at least once daily during the entire 21 days but twice daily during peak mortality periods. In pathogenesis experiments, samples of the lung, liver, kidney, and spleen were obtained from three mice per treatment group, as described previously (21, 22), after euthanasia using carbon dioxide inhalation on days 1, 3, 5, 7, 10, 12, or 14 following CV or VV WR infections. Pooled organ samples were homogenized in medium (10% wt/vol) and frozen at −70°C until assayed for virus.

Virus quantitation. Samples were thawed and assayed on Vero cells using an agarose overlay plaque assay to determine VV or CV titers (21, 22). Briefly, samples of organ homogenates were diluted serially, and a 0.2-ml volume was placed into each of 12 wells of Vero cell monolayers and incubated for 1 h. A 0.5% agarose in minimal essential medium (SeaKem, ME agarose; FMC BioProducts, Rockland, ME) solution was added to each well, and the cultures were incubated for 3 days. Cultures were stained with neutral red (Gibco, Rockville, MD) for approximately 6 h prior to the enumeration of viral plaques.

Statistical evaluations. Mortality rates were analyzed by Fisher's exact test (two-tailed) and mean day of death and virus titers in tissues by using Mann-Whitney U rank sum test (two-tailed). A P value of ≤ 0.05 was considered significant.

RESULTS

In vitro activity of ether lipid esters of CDV. We have reported previously that CDV has a required concentration of 25 to 50 μM to effectively reach the EC_{50} against CV and VV in HFF cells (14, 18). For comparison purposes, the results for CDV and HDP-CDV are included with the results for (*S*)-HPMPA, HDP-(*S*)-HPMPA, and ODE-(*S*)-HPMPA in Table 1. Efficacy (VV Copenhagen and CV) and cytotoxicity data for (*S*)-HPMPA, HDP-(*S*)-HPMPA, and ODE-(*S*)-HPMPA were reported previously (3), while efficacy against VV WR was determined in this study. The EC_{50} of the parent (*S*)-HPMPA was 2.7 μM for VV Copenhagen, 6.5 μM for VV WR, and 4.0 μM for CV Brighton. The EC_{50} for HDP-(*S*)-HPMPA was 0.01 μM for VV Copenhagen, 0.3 μM for VV WR, and 0.02 μM for CV. Similar activity was observed for ODE-(*S*)-HPMPA. Both of the lipid analogs were approximately 65- to 300-fold more active against VV and CV than unmodified (*S*)-HPMPA, 4- to 80-fold more active than HDP-CDV, and 3,000-fold more active than CDV. The evaluation of compound toxicity is a key component of drug development, and while the prodrugs were more toxic than the parent compound, the resulting selectivity index (SI; CC_{50}/EC_{50}) values for VV Copenhagen and CV Brighton were up to ninefold higher than that for the parent compound. On the contrary, the higher prodrug EC_{50} values presented against VV-WR resulted in an SI value for (*S*)-HPMPA that was greater than those of both lipid analogs.

TABLE 1. Efficacy and cytotoxicity of alkoxyalkyl esters of (*S*)-HPMPA

Compound	CC_{50}[a] (μM)	VV WR		VV Copenhagen		CV Brighton	
		EC_{50}[a] (μM)	SI	EC_{50} (μM)	SI	EC_{50} (μM)	SI
CDV	>317 ± 0	29 ± 3.1	>10.9	26 ± 5.2	>12.2	35 ± 4.3	>9.1
HDP-CDV[b]	31 ± 2.1	1.1 ± 1.0	28	0.8 ± 0.4	39	0.6 ± 0.3	52
(*S*)-HPMPA	>289 ± 38[c]	6.5 ± 1.6	>44	2.7 ± 2.4[c]	>107[c]	4.0 ± 3.8[c]	>72[c]
HDP-(*S*)-HPMPA	9.7 ± 6.2[c]	0.3 ± 0.2	32	0.01 ± 0.004[c]	970[c]	0.02 ± 0.006[c]	485[c]
ODE-(*S*)-HPMPA	1.5 ± 0.4[c]	0.1 ± 0.06	15	0.01 ± 0.003[c]	150[c]	0.02 ± 0.02[c]	75[c]

[a] Values are the means from two or more assays ± standard deviations.
[b] Values were reported previously (18).
[c] Value was reported previously (3).

Pharmacokinetics. The plasma drug levels following a 10 mg/kg dose of HDP-(*S*)-HPMPA given orally or i.p. are shown in Fig. 2. The maximum concentration of the drug in plasma (C_{max}) was 1.1 μM at 1 h in the oral group compared to 2.2 μM at 3 h in the i.p. group (Table 2). Plasma HDP-(*S*)-HPMPA and metabolites declined rapidly, reaching 0.03 μM in the oral group and 0.08 μM in the i.p. group at 24 h. The half-life ($t_{1/2}$) for oral HDP-(*S*)-HPMPA was 11.5 h versus 2 h for the i.p.-administered drug. The plasma area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) values for oral and i.p. HDP-(*S*)-HPMPA were 12.1 and 16.4 nmol · h/g, respectively. Based on the comparative AUC values for oral and i.p. HDP-(*S*)-HPMPA, we estimate the relative oral bioavailability for HDP-(S)-HPMPA to be 74% in mice (Table 2). The relative oral bioavailability of (*S*)-HPMPA is 24% (K. Hostetler, unpublished data).

i.p. administration of HDP-(*S*)-HPMPA gave higher drug levels in the organs than those noted with oral administration. Peak tissue levels were highest in the liver, at 130.0 nmol/g at 1 h for i.p. compared to 21.0 nmol/g at 3 h for oral administration (data not shown). There were also higher peak levels for the kidney and lung in the i.p. group than in the oral group. Drug exposure in the kidney was notably lower with oral administration of HDP-(*S*)-HPMPA than with i.p. administration. The peak level in the kidney was 23.0 nmol/g at 1 h for the i.p. group compared to 5.1 nmol/g at 3 h for the oral group. Drug levels in the lung peaked at 2.8 nmol/g at 1 h for the i.p. group compared to 1.9 nmol/g at 3 h for the oral group (data not shown). Although tissue levels of total HDP-(*S*)-HPMPA and metabolites in some tissues are low, the EC_{50} of HDP-(*S*)-HPMPA for VV and CV is only 0.01 to 0.02 μM, suggesting that the observed tissue drug levels are sufficient to inhibit viral replication.

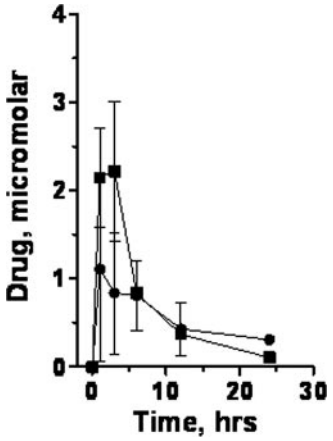

FIG. 2. Plasma levels of HDP-(*S*)-HPMPA and metabolites in mice after oral (●) or i.p. (■) administration. Error bars represent standard deviations from three experiments.

Activity of HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA in VV infections of mice. Compounds were dissolved in deionized water and administered by oral gavage once daily at doses of 30, 10, or 3 mg/kg for 5 consecutive days to infected mice beginning 24, 48, or 72 h after viral inoculation. CDV was administered i.p. at 10 or 15 mg/kg as the positive control. No toxicity was associated with the 30-mg/kg dose of HDP-(*S*)-HPMPA or ODE-(*S*)-HPMPA, and each compound significantly reduced final mortality ($P < 0.01$) at one or more times of initiation of therapy (Tables 3 and 4). HDP-(*S*)-HPMPA significantly reduced mortality rates when treatment was initiated as late as 72 h after viral inoculation with 30 or 10 mg/kg. Treatment with the 3-mg/kg dose was not effective if treatment was delayed until 72 h after infection (Table 3). The results of treatment with ODE-(*S*)-HPMPA on VV infection of mice are summarized in Table 4. In the vehicle-treated group, there was 67 to 100% mortality, whereas in the CDV-treated mice, all survived even when treatment was delayed until 72 h after infection. In mice treated with ODE-(*S*)-HPMPA, significant protection was observed at 30, 10, or 3 mg/kg when treatment was initiated as late as 48 h postinfection. Due to the low level of mortality in the control group (67%), a significant effect could not be demonstrated with ODE-(*S*)-HPMPA at 72 h postinfection.

Activity of HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA in CV infections of mice. Compounds were dissolved in deionized water at doses of 30, 10, or 3 mg/kg and administered once daily for 5 consecutive days by oral gavage to infected mice beginning 24, 48, or 72 h after viral inoculation. CDV was administered i.p. at 10 mg/kg as a positive control. At 30 and 10 mg/kg, HDP-(*S*)-HPMPA significantly reduced final mortality

TABLE 2. Pharmacokinetic parameters for HDP-(*S*)-HPMPA[a]

HDP-(*S*)-HPMPA administration	$t_{1/2}$ (h)	C_{max} (μM)	T_{max} (h)	AUC (nmol · h/g)	% Oral bioavailability
i.p.	2.5	2.2	3	16.4	
Oral	11.5	1.1	1	12.1	74

[a] T_{max}, time to maximum concentration of drug in serum.

TABLE 3. Effect of once-daily oral treatment with HDP-(*S*)-HPMPA on mortality of BALB/c mice infected with VV WR

Time of treatment and dosage[a]	No. of mice that died/ total no. of mice (%)	*P* value	MDD ± SD[b]	*P* value
24 h				
Vehicle-water	12/15 (80)		8.3 ± 1.0	
CDV, 10 mg/kg	0/15 (0)	<0.001		
HDP-(*S*)-HPMPA				
30 mg/kg	2/15 (13)	0.001	7.0 ± 1.4	NS[c]
10 mg/kg	0/15 (0)	<0.001		
3 mg/kg	4/15 (27)	<0.01	7.0 ± 1.4	NS
48 h				
Vehicle-water	11/15 (73)		8.5 ± 1.4	
CDV, 10 mg/kg	0/15 (0)	<0.001		
HDP-(*S*)-HPMPA				
30 mg/kg	1/15 (7)	<0.001	11.0 ± 0	0.09
10 mg/kg	2/15 (13)	<0.01	8.0 ± 0	NS
3 mg/kg	5/15 (33)	0.07	9.2 ± 2.2	NS
72 h				
Vehicle-water	15/15 (100)		8.3 ± 2.6	
CDV, 10 mg/kg	0/15 (0)	<0.001		
HDP-(*S*)-HPMPA				
30 mg/kg	1/15 (7)	<0.001	8.0 ± 0	NS
10 mg/kg	8/15 (53)	<0.01	9.1 ± 2.0	0.06
3 mg/kg	13/15 (87)	NS	7.8 ± 0.7	NS

[a] Treatments commenced at indicated times postinoculation and continued for 5 days. All treatments were given orally except for CDV, which was administered i.p. beginning 24, 48, or 72 h after viral inoculation.
[b] MDD ± SD, mean day to death ± standard deviation.
[c] NS, not significant.

rates ($P < 0.01$) at 24 or 48 h postinoculation (Table 5). Only the 30-mg/kg dose exhibited activity when treatment was initiated 72 h postinoculation. The positive control, CDV, was highly effective ($P < 0.001$) at all dosages and all times, again providing 100% protection rates when given as late as 72 h postinoculation. The effect of treatment with ODE-(*S*)-HPMPA on mortality of mice inoculated with CV is summarized in Table 6. Significant protection was observed with 30

TABLE 4. Effect of once-daily oral treatment with ODE-(*S*)-HPMPA on mortality of BALB/c mice infected with VV-WR

Time of treatment and dosage[a]	No. of mice that died/ total no. of mice (%)	*P* value	MDD ± SD[b]	*P* value
24 h				
Vehicle-water	15/15 (100)		7.7 ± 0.7	
CDV, 15 mg/kg	0/15 (0)	<0.001		
ODE-(*S*)-HPMPA				
30 mg/kg	2/15 (27)	<0.001	8.3 ± 1.4	NS[c]
10 mg/kg	0/15 (0)	<0.001		
3 mg/kg	10/15 (67)	<0.05	8.8 ± 0.9	<0.01
48 h				
Vehicle-water	15/15 (100)		7.5 ± 0.6	
CDV, 15 mg/kg	0/15 (0)	<0.001		
ODE-(*S*)-HPMPA				
30 mg/kg	6/15 (40)	<0.01	9.7 ± 0.8	<0.01
10 mg/kg	3/15 (20)	<0.001	10.3 ± 3.1	NS
3 mg/kg	8/15 (53)	<0.01	7.9 ± 0.6	NS
72 h				
Vehicle-water	10/15 (67)		8.5 ± 1.6	
CDV, 15 mg/kg	0/15 (0)	<0.001		
ODE-(*S*)-HPMPA				
30 mg/kg	11/15 (73)	NS	10.5 ± 0.8	<0.01
10 mg/kg	5/15 (33)	NS	9.0 ± 2.8	NS
3 mg/kg	6/15 (40)	NS	9.0 ± 2.1	NS

[a] Treatments commenced at indicated times postinoculation and continued for 5 days. All treatments were given orally except for CDV, which was administered i.p. beginning 24, 48, or 72 h after viral inoculation.
[b] MDD ± SD, mean day to death ± standard deviation.
[c] NS, not significant.

TABLE 5. Effect of once-daily oral treatment with HDP-(*S*)-HPMPA on mortality of BALB/c mice infected with CV-BR

Time of treatment and dosage[a]	No. of mice that died/ total no. of mice (%)	*P* value	MDD ± SD[b]	*P* value
24 h				
Vehicle-water	15/15 (100)		10.7 ± 1.9	
CDV, 10 mg/kg	0/15 (0)	<0.001		
HDP-(*S*)-HPMPA				
30 mg/kg	2/15 (13)	<0.001	15.0 ± 2.8	<0.05
10 mg/kg	10/15 (67)	<0.05	13.8 ± 3.4	<0.05
3 mg/kg	14/15 (93)	NS[c]	11.4 ± 1.5	<0.05
48 h				
Vehicle-water	15/15 (100)		10.7 ± 2.3	
CDV, 10 mg/kg	0/15 (0)	<0.001		
HDP-(*S*)-HPMPA				
30 mg/kg	2/15 (13)	<0.001	16.5 ± 4.2	0.05
10 mg/kg	4/15 (27)	<0.001	12.3 ± 6.1	NS
3 mg/kg	13/15 (87)	NS	12.7 ± 2.2	<0.01
72 h				
Vehicle-water	15/15 (100)		10.7 ± 1.2	
CDV, 10 mg/kg	0/15 (0)	<0.001		
HDP-(*S*)-HPMPA				
30 mg/kg	2/15 (13)	<0.001	6.5 ± 0.7	NS
10 mg/kg	11/15 (73)	NS	12.3 ± 2.8	0.05
3 mg/kg	13/15 (87)	NS	10.1 ± 1.7	NS

[a] Treatments commenced at indicated times postinoculation and continued for 5 days. All treatments were given orally except for CDV, which was administered i.p. beginning 24, 48, or 72 h after viral inoculation.
[b] MDD ± SD, mean day to death ± standard deviation.
[c] NS, not significant.

and 10 mg/kg when begun 24, 48, or 72 h postinoculation, but the 3 mg/kg dose was active only at 24 h postinoculation.

Effect of HDP-(*S*)-HPMPA or ODE-(*S*)-HPMPA on the pathogenesis of VV or CV infections of mice. To determine the effect of treatment with HDP-(*S*)-HPMPA or ODE-(*S*)-HPMPA on the replication of VV in target organs of mice, animals were inoculated with VV and treated orally with 30 mg/kg of each of the compounds once daily for 5 days begin-

TABLE 6. Effect of once-daily oral treatment with ODE-(*S*)-HPMPA on mortality of BALB/c mice infected with CV-BR

Time of treatment and dosage[a]	No. of mice that died/ total no. of mice (%)	*P* value	MDD ± SD[b]	*P* value
24 h				
Vehicle-water	15/15 (100)		8.4 ± 0.9	
CDV 10 mg/kg	0/15 (0)	<0.001		
ODE-(*S*)-HPMPA				
30 mg/kg	1/15 (7)	<0.001	13.0 ± 0	NS[c]
10 mg/kg	1/15 (7)	<0.001	14.0 ± 0	NS
3 mg/kg	9/15 (60)	<0.05	13.7 ± 3.6	<0.001
48 h				
Vehicle-water	15/15 (100)		9.6 ± 0.7	
CDV 10 mg/kg	2/15 (13)	<0.001	11.0 ± 1.4	<0.05
ODE-(*S*)-HPMPA				
30 mg/kg	2/15 (13)	<0.001	9.5 ± 0.7	NS
10 mg/kg	3/15 (20)	<0.001	11.0 ± 5.7	NS
3 mg/kg	12/15 (80)	NS	10.6 ± 1.9	<0.05
72 h				
Vehicle-water	15/15 (100)		9.9 ± 1.2	
CDV 10 mg/kg	0/15 (0)	<0.001		
ODE-(*S*)-HPMPA				
30 mg/kg	5/15 (33)	<0.001	10.6 ± 1.1	NS
10 mg/kg	10/15 (67)	<0.05	12.2 ± 2.9	<0.05
3 mg/kg	14/15 (93)	NS	11.1 ± 2.3	NS

[a] Treatments commenced at indicated times postinoculation and continued for 5 days. All treatments were given orally except for CDV, which was administered i.p. beginning 24, 48, or 72 h after viral inoculation.
[b] MDD ± SD, mean day to death ± standard deviation.
[c] NS, not significant.

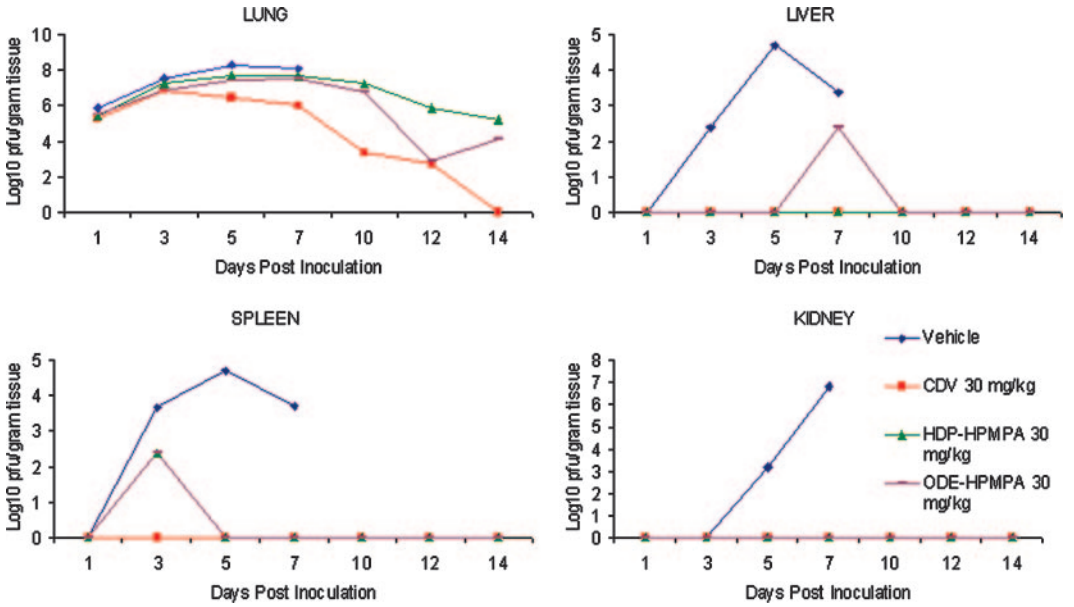

FIG. 3. Effect of daily oral treatment with HDP-(*S*)-HPMPA or ODE-(*S*)-HPMPA on the pathogenesis of VV infection in mice. Treatment with 30 mg/kg was initiated 24 h after viral inoculation and continued once daily for 5 consecutive days. Points represent mean $\log_{10}$ PFU/gram of tissue.

ning 24 h after infection. CDV was included as the positive control. On various days postinfection, animals were euthanized, and their tissues were removed and assayed for VV. Treatment with both analogs resulted in a significant reduction in mortality. There was also a 4 to 5 $\log_{10}$ decrease in virus titers for the liver, spleen, and kidney. Consistent with previous studies using other antiviral therapies, there was little alteration in virus titers for the lung, although all mice treated with either HDP-(*S*)-HPMPA or ODE-(*S*)-HPMPA survived. Our prior work has shown that lung titers of virus do not correlate with death due to disease, with survival being more closely correlated to reductions in virus titers for the liver, spleen, and kidney. In the group that received i.p. CDV, virus titers for lung tissue were reduced after day 5 and the virus appeared to clear more rapidly (Fig. 3).

Similar studies were performed to determine the effect of these compounds on the replication of CV in tissues. HDP-(*S*)-HPMPA or ODE-(*S*)-HPMPA was given at a dose of 30 mg/kg once daily for 5 days beginning 24 h after viral inoculation. Both HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA reduced

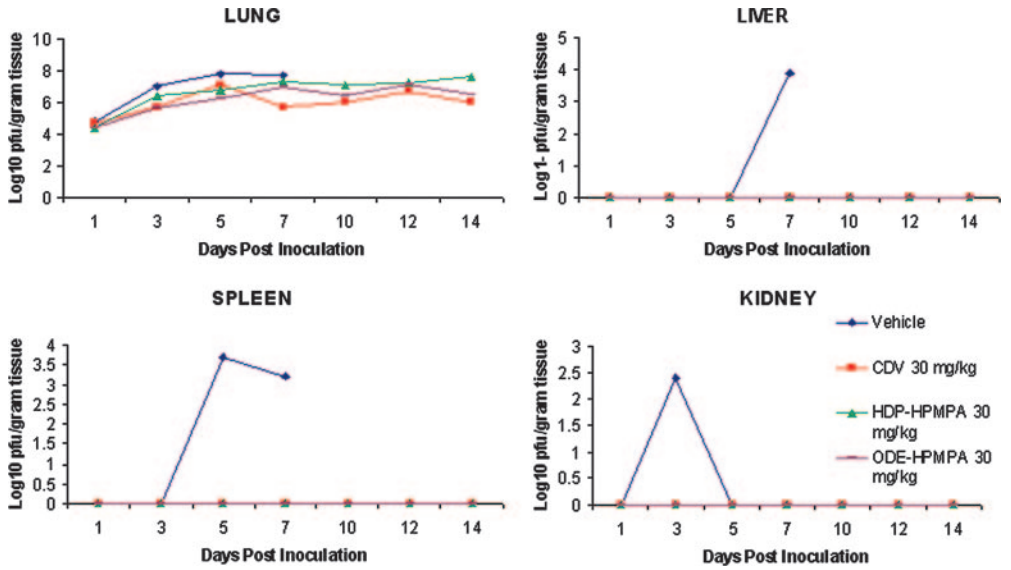

FIG. 4. Effect of daily oral treatment with HDP-(*S*)-HPMPA or ODE-(*S*)-HPMPA on the pathogenesis of CV infection in mice. Treatment with 30 mg/kg was initiated 24 h after viral inoculation and continued once daily for five consecutive days. Points represent mean $\log_{10}$ PFU/gram of tissue.

viral replication by 2.5 to 4.0 $\log_{10}$ in the liver, spleen, and kidney of CV-infected mice (Fig. 4). As with the VV infection, lung titers remained unaffected, although all treated mice survived. Again, similar results were obtained with CDV given i.p.

DISCUSSION

We have shown previously that (*S*)-HPMPA is a highly active antiviral in poxvirus- and CMV-infected HFF cells when converted to its HDP or ODE esters (3). Recently, HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA have also been shown to be active against VV and CV in HEL cells and organotypic epithelial raft cultures, with SI values from 435 to 0.3, respectively (19, 24), and against a number of viruses in lamb keratinocytes and HEL cells (SI values from 40 to 3,290, respectively) (8). Comparatively, our in vitro studies reveal SI values from 15 to 970, with HDP-(*S*)-HPMPA appearing more efficacious, whereas ODE-(*S*)-HPMPA emerged as the more active compound overall in previously published literature, with antiviral activity 30 to 15,300 times greater than that of CDV (3, 8, 19).

In this report, we have demonstrated that HDP-(*S*)-HPMPA is orally active, in contrast to unmodified acyclic nucleoside phosphonates. The relative oral bioavailability of HDP-(*S*)-HPMPA is 74%, providing plasma and tissue levels of drug sufficient to inhibit replication of VV and CV both in vitro (EC_{50}, 0.01 to 0.3 μM) and in vivo. Plasma levels had peak values at 1 h for the oral group and at 1 to 3 h for the i.p. group. After 6 h, drug levels for the two routes of administration decline at similar rates. All organs had greater drug AUC values with i.p. than with oral administration, with the liver having the highest drug AUC value. It is important that renal exposure is lowered with oral administration, thereby minimizing possible nephrotoxicity, a problem with parenteral administration of CDV and (*S*)-HPMPA. Similar findings were demonstrated when HDP-CDV was synthesized and compared with CDV when evaluated for bioavailability, renal toxicity, and efficacy against orthopoxvirus and herpesviruses (6, 17, 22, 26).

HDP-(*S*)-HPMPA given orally 72 h after intranasal challenge with either VV or CV at a dose of 30 mg/kg daily for 5 days provided nearly complete protection against mortality without any apparent toxic effects in mice. Previous studies with i.p. (*S*)-HPMPA indicated that nephrotoxicity was a major dose-limiting toxicity (4, 23). Oral administration of (*S*)-HPMPA was 20 times less active than parenteral administration against VV infections in mice (10). In those studies, doses of up to 100 mg/kg/day were utilized with no mention of toxicity (10). We also did not observe toxicity in these studies with oral HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA. ODE-(*S*)-HPMPA, given 72 h after CV infection or 24 to 48 h after VV infection, also provided significant protection from mortality. Both compounds have potential utility for the treatment of human cases of adverse reactions to smallpox vaccination or for the treatment of monkeypox or smallpox. Additional studies are needed to determine if HDP-(*S*)-HPMPA or ODE-(*S*)-HPMPA are synergistic with other anti-orthopoxvirus agents, such as ST-246 (27). If synergy with other such antivirals can be demonstrated, it is possible that reduced dosages of either or both drugs could widen the margin of safety.

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